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THE MICROBIOLOGICAL
FORMATION OF IRON SULPHIDES

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3. The microbiological formation of iron sulphides

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Abstract.—An experimental study of the formation of iron sulphides by the bacterium *Desulfovibrio desulfuricans* strain Canet 41 resulted in the formation of five iron sulphide minerals: greigite, mackinawite, marcasite, pyrite and pyrrhotite. Smythite was not detected. The iron salts sulphidised in the chemically-defined media were a complex ferroso-ferric oxyhydroxide, 'green rust 2', and synthetic goethite. Complete x-ray diffraction data are given for 'green rust 2' based on a hexagonal unit cell with $a_0 = 3.17$ and $c_0 = 10.94$ Å.

Comparison with the results from inorganic experimentation reveal no crystallochemical differences between biogenic and abiogenic sulphides. The mechanisms of formation are the same in each case. No textural differences were observed apart from the apparent flotation of the pyrite in cultures containing large quantities of oil-like material from the decomposition of bacteria and mucin.

The effects of microbiological carbon dioxide production and the presence of large amounts of organic matter in the microorganic environment are examined and discussed.

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INTRODUCTION

Since the work of MILLER (1950) several investigators have examined the metal sulphides produced through the activities of sulphate-reducing bacteria. MILLER (1950), BAAS BECKING and MOORE (1960), and TEMPLE (1964) produced iron, copper, zinc and silver sulphides using pure and raw cultures of sulphate-reducers. BERNER (1964) investigated the iron sulphides (particularly mackinawite) formed by the action of raw cultures on iron salts. FREKE and TATE (1961) obtained a material which they identified as 'magnetic Fe_3S_4 ' from pure cultures in a semi-continuous cultures apparatus. The author (D. R.) has examined their material by means of x-ray powder diffraction analysis; the material was found to be a mixture of greigite (Fe_3S_4), goethite and hematite.

However, although it has been demonstrated that sulphate-reducing bacteria can be intimately involved with the production of metal sulphides, the detailed nature of these sulphides has not been examined. By examining these biogenic sulphides and comparing them with abiologic sulphides certain information can be deduced regarding the role played by the organism and the possibility of differentiating between mineral sulphides of the two origins. As a result of the detailed work recently published by RICKARD (1969) on the chemistry of the iron sulphides at low temperatures and pressures in aqueous solutions, it is now possible to compare those iron sulphides produced bacteriologically with those produced abiologically in order to examine whether there is any crystallochemical or textural difference between them. This paper reports such an investigation.

EXPERIMENTAL

Organism

The organism used in these experiments was *Desulfovibrio desulfuricans*, strain Canet 41. (N. C. I. B. 8393). This strain was originally isolated from Etang de Canet, Perpignan, France (MACPHERSON and MILLER, 1963). The main reason for the choice of Canet 41 as the experimental organism was its extraordinary salt tolerance. This strain grows at salt concentrations of 0-12 per cent (LITTLEWOOD and POSTGATE, 1967) and it was shown to withstand sudden changes from 0 per cent to 3 per cent NaCl during this experimentation. The optimum pH of the organism depends on the nature of its environment. Although under ideal conditions it is about $\text{pH} = 7.2$, this investigation showed that the organism became more resistant to extremes of conditions at $\text{pH} = 8$

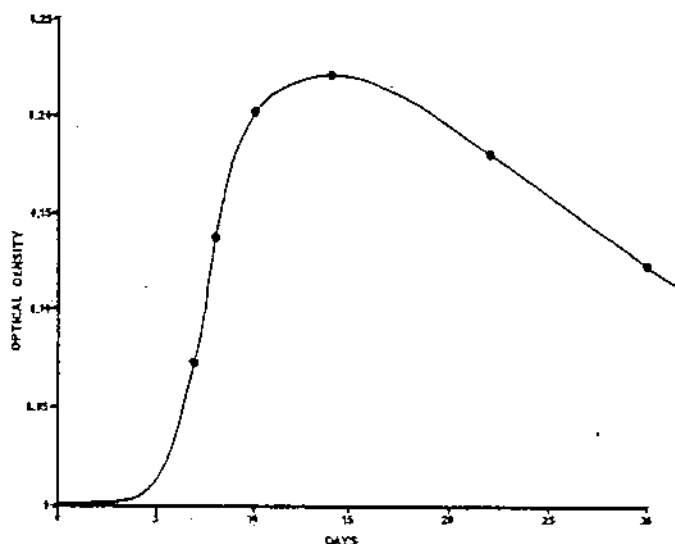


Fig. 1. Growth curve of *Desulfovibrio desulfuricans* strain Canet 41.

and above, than in the more acid media. Canet 41 can grow at temperatures from 0–65° C, but its optimum temperature in artificial media is about 30° C.

Although it has a typical vibrio form, it is inclined to pellicular growth. The formation of a pellicle is the cause of many experimental difficulties, especially with regard to counting and inoculation. It was found that the dilution and anaerobic plating methods of counting were very unreliable. Measurements of optical density of suspensions of bacteria produced through the dispersion of the pellicle (by vigorous agitation in the presence of sodium hexametaphosphate) were found to be proportional to the dry weight of bacteria plus pellicle. The optical density method could therefore be used for the measurement of relative organic production, and fig. 1 shows a typical growth curve for *Desulfovibrio desulfuricans* strain Canet 41. The curve is similar to growth curves obtained from other strains of sulphate-reducing bacteria (SENEZ, 1951 and MACPHERSON and MILLER, 1963), and is characterised by a rapid linear growth phase and a short stationary phase. The shortness of the stationary phase and the linearity of the following decline is caused by the decomposition of the pellicle and cell autolysis. The final result is an equilibrium between autolysis and growth, and Canet 41 can be recultured after more than one year of cultivation.

However, even optical density measurements could not be used on cultures grown in the presence of iron salts because of the spurious readings given by the iron sulphide product admixed with the pellicle and suspended in the medium. Counting was therefore not employed, and growth was determined

qualitatively according to hydrogen sulphide production. Since it was impossible to obtain a homogeneous suspension of the bacteria for inoculation, a large amount of inoculum was employed: 1 ml inoculum per 25 ml medium.

Media

The growth characteristics of sulphate-reducing bacteria are dependent on the conditions of cultivation, and, although Canet 41 is far more tolerant of excessive conditions than most other strains, it is still susceptible to media variations. Pellicular growth is probably one reflection of this susceptibility.

In the original experimental plan it was decided that, in order to simplify the interpretation of results, strict chemical controls should be employed. To this end, a chemically-defined medium had to be developed to support the growth of Canet 41.

The medium used as a base was that of MACPHERSON and MILLER (1963) (Table 1 (a)) which was preferred to the only other chemically-defined medium developed for the growth of sulphate-reducers because of the latter's requirement of the addition of mixed amino acids and adenosine-triphosphate (KADOTA and MIYOSHI, 1960).

Table 1. *Chemically-defined media*

(a) MACPHERSON and MILLER (1963)		(b) 0100		(c) 0200	
Component ¹	mM	Component ¹	mM	Component ¹	mM
Lactic acid	100	Lactic acid	100	Lactic acid	240
KH ₂ PO ₄	2.5	KH ₂ PO ₄	2.5	KH ₂ PO ₄	2.5
NH ₄ Cl	10	(NH ₄) ₂ SO ₄	50	(NH ₄) ₂ SO ₄	50
Na ₂ SO ₄	50	CaCl ₂	0.5	CaCl ₂	0.5
CaCl ₂	0.5	MgSO ₄ · 7H ₂ O	0.25	MgSO ₄ · 7H ₂ O	0.25
MgSO ₄ · 7H ₂ O	0.25	NaCl	430	NaCl	430
+ metal solution		+ thioglycollic acid ²		+ thioglycollic acid ²	
+ Na ₂ S					

¹ Analytical grade reagents.

² To —200mV.

It was anticipated that high electrolyte concentration might affect the nature of any precipitate formed in the medium, and therefore the basal medium was modified by the addition of 2.5 per cent (0.43 M) sodium chloride. Sodium sulphate was replaced by an equal molar concentration of ammonium sulphate, a weaker base, since with the conversion of about 50 mM

sodium sulphate to sodium sulphide, a large shift of pH to the alkaline side would have been inevitable. The presence of ammonium sulphate and sodium chloride made it unnecessary to add ammonium chloride.

A metal solution containing trace amounts of boron, cobalt, manganese, molybdenum and zinc was not included since there are conflicting data on the necessity of these elements for bacterial growth (HATA, 1960a, 1960b, KIMATA *et al.*, 1955 and POSTGATE, 1965). It is possible that trace element requirements are interdependent through the ability of one element to substitute for another, and may differ for individual strains.

It has been established that *D. desulfuricans* has a minimum iron requirement for optimum growth (POSTGATE, 1956). For example, the obligate halophile EL Agheila Z, required a minimum of 100 $\mu\text{MFe/ml}$. The iron content of the final medium, without the addition of discrete iron salts, was analysed spectrophotometrically by the 2-2' dipyridyl method (MOSS and MELLOR, 1942). The medium was found to contain an average of 400 $\mu\text{MFe/ml}$. The impurity specifications of the analytical grade reagents employed showed that up to 800 $\mu\text{MFe/ml}$ could be expected.

Finally, sodium sulphide, which is used as a reducing agent in the basal medium, was regarded as undesirable since it would react to give iron sulphides in its own right. It was replaced by thioglycollic acid. This was preferred to cysteine, which can act as a nutrient to such well-known bacteria as *Escherichia coli*, and other reducing agents, since it is more thermotolerant and may be autoclaved.

The final medium (Medium 0100, Table 1 (b)) gave reasonable growth on repeated subculture with or without sodium chloride.

For the iron-rich media, the amount of iron required was calculated from the premise that about 200 mg FeS (theoretical) would be required for analysis per 25 ml solution. 13.95 g ferrous sulphate and 16.10 g Mohr's salt per litre were found to give the necessary 182 mM iron concentration. The iron: sulphur ratio of the medium was then about 1:2, allowing the possible formation of FeS_2 . In medium 2100, synthetic goethite was used in place of the ferrous salts, to the same iron concentration. Details of the preparation of synthetic goethite have been given elsewhere (RICKARD, 1969).

These initial experiments gave rather poor sulphide yields since the lactate concentration was kept low, because it had been suggested that increased lactate concentration decreased the growth rate (MACPHERSON and MILLER, 1963). This was finally overcome in the 1200 and 2200 series of experiments by increasing the lactate concentration, whilst at the same time maintaining the growth rate at its previous level, by growing the stock cultures in the high-lactate iron-free medium 0200 with 1 g yeast extract added per litre. It was calculated that only 0.004 per cent yeast extract would be carried over in the inoculum into the experimental cultures, assuming a total lack of transforma-

tion of the yeast extract in the stock cultures. Chemically, this is a negligible concentration.

Shake and plate cultures in glucose-peptone-yeast extract-sulphate medium were used for contamination checks, as recommended by POSTGATE (1951). Both deep shake and aerobic plate checks were made. In fact, Canet 41 does not grow well on this medium, and the complete absence of growth had to be regarded as an indication of a lack of contaminants. Throughout this investigation contaminants were rarely discovered, and were generally limited to fungi.

Methods

The bacteria were cultivated in batch cultures containing 25 ml of medium. Medium 100 and 1200 were prepared by adding ferrous salts to Medium 0100 and 0200. The pH of the medium at this stage was about 3, preventing iron precipitation. The media were autoclaved at 15 psi and 121° C for 15 minutes. The pH was poised at $\text{pH} = 6.3 \pm 0.3$, 7.2 ± 0.2 and 8.1 ± 0.1 and three replicas were made at each pH. The Eh was poised to -200 mV with thioglycollic acid. The poisoning of the pH and Eh at these values resulted in the formation of iron oxyhydroxides. Experiments with *Aspergillus niger* and metal-complexing agents (CHOUDHARY and PIRT, 1965) have shown that the use of these agents in large concentrations such as would be necessary to keep all the iron in these experiments in solution, is toxic to bacteria and results in the dissolution of the cell walls.

The poised media were inoculated with 1 ml of inoculum from a young, vigorously growing stock culture (usually 4 to 5 days old), grown in Medium 0100 or 0200. The rimless pyrex test-tubes containing 25 ml of inoculated media were placed in anaerobic jars under an atmosphere of oxygen-free nitrogen.

Essentially, the methods employed in the preparation and inoculation of the goethite-containing Medium 2200 were identical, except that a precipitate was present before autoclaving, when the pH was about 6.

The bacteria were cultivated for periods ranging between 2 weeks and 9 months, and replicas were taken at each time interval examined. The method of examination of the products has been described elsewhere (RICKARD, 1969) and included x-ray powder diffraction analysis, polished section microscopy and chemical analysis.

PRELIMINARY RESULTS

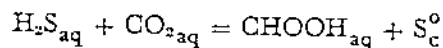
Two peripheral investigations had to be performed before the results from the sulphidation experiments could be analysed

The effects of bacterial activity on the pH of the medium

The sulphate-reducing bacteria produce two major compounds during the metabolism of lactate: sulphide (as hydrogen sulphide, bisulphide ions or sulphide ions) and carbon dioxide (as carbon dioxide, bicarbonate or carbonate ions). As was pointed out above the effect of the transformation of ammonium sulphate to ammonium sulphide results in alkalisation of the medium. However, much of this sulphide is fixed in the culture as iron sulphide, in the presence of large concentrations of iron salts.

The action of carbon dioxide is more complex. GARRELS & CHRIST (1965) showed that the sulphide activity must be extremely low in the presence of iron salts and carbonate, before iron carbonate will precipitate in the place of iron sulphides. In these experiments no carbonate salts were identified in the final products, which is consistent with these theoretical conclusions. It is possible that iron carbonates were formed initially and subsequently sulphidised, but no evidence was found to support this contention.

The production of carbon dioxide could have some indirect effect by modifying or reacting with the bacteriologically-produced sulphide species. The reaction between carbon dioxide and hydrogen sulphide is well known in certain microorganisms, especially the sulphide-oxidising photosynthetic autotrophs. The overall reaction performed by organisms of this type is:



However, this reaction has its equilibrium strongly on the left-hand side because of the very large, negative ΔF° of aqueous CO_2 (-92.31 kcal) and the small negative or positive ΔF° of the dissolved sulphide species. As the carbon number of the organic product increases, the number of carbon dioxide molecules required also increases, and therefore increasing complexity of the product results in the reaction equilibrium moving further to the left-hand side of the equation. Carbon bisulphide cannot form in large quantities during this reaction because of its large positive ΔF° ($+15.20$ kcal).

The sulphide-oxidising microorganisms are able to carry out this type of reaction by the addition to the system of the energy accrued during photosynthesis. Six to nine quanta of light per molecule of product are required, depending on the wavelength. Equilibrium in such a system is rapidly reached, being catalysed enzymatically.

The theoretical conclusion that no reaction occurs abiologically at 25°C was confirmed experimentally. Carbon dioxide gas was blown through an agitated sealed vessel containing molar sodium sulphide and oxygen-free nitrogen. The Eh of the system was recorded on a chart recorder over a period of two days. All the carbon dioxide dissolved was recovered by gravimetric analysis using strontium chloride.

However the experiment was accompanied by a pH change from > 14 to 10 and a rise in Eh from -570 to -370 mV. The action of carbon dioxide was therefore to alter the pH, thereby effecting a transformation of the couples producing the Eh. Since the activity ratios of the dissolved sulphur species were not unity, it is impossible to predict precisely which redox couples were operative. However it is definitely known that a change from dominant sulphide ion to dominant bisulphide ion does occur at about $\text{pH} = 14$.

Because of the delicate interrelationships of the various sulphur species, especially in those areas coincident with environments where most of these bacteriological reactions occur, ($\text{pH} = 6-9$, $\text{Eh} = 0$ to -400 mV), small variations in pH, such as that caused by the production of carbon dioxide, may cause major changes in the sulphur species present in solution.

There are therefore two opposing pH effects of bacterial metabolism in these media. Firstly there is a tendency for alkalisation through the production of ammonium sulphide by that part of the sulphide not involved in the formation of iron sulphides. Secondly there is an acidification caused by the production of carbon dioxide. The overall effect of these opposing actions is to initially decrease the pH as sulphide is fixed as an iron sulphide; subsequently, when all the iron is sulphidised, the pH gradually increases once more. In the media described above, the rate and degree of these changes are limited by the buffering effect of the various ionic equilibria involved. In particular, the HCO_3^- - H_2CO_3 equilibrium (at about $\text{pH} = 6.4$) and the H_2S - HS^- equilibrium (about $\text{pH} = 7$) will be important rate controlling factors in these changes in pH.

The nature of the initial precipitate in the ferrous iron-containing media

It has been shown (BERNER, 1964 and RICKARD, 1969) that the nature of the iron salt being sulphidised can have an extremely important effect on the iron sulphide product. At the initial pH of the media ferrous iron precipitates. This precipitate is white ferrous hydroxide. As pointed out by GARRELS (1959), ferrous hydroxide reacts with water to form, in the long run, magnetite. The exact nature of these transformations has been described by BERNAL, DASGUPTA and MACKAY (1958), and RICKARD (1968). Initially the ferrous hydroxide changes to relatively stable complex ferroso-ferric oxyhydroxides known as the 'green rusts'. There are two main types of 'green rust' encountered in these experiments: 'green rust 1' and 'green rust 2'. Both are dark blue-green hexagonal materials with indefinite formulae, which may contain a variable amount of ferric iron. 'Green rust 2' is far more stable than 'green rust 1' and resists air-oxidation for up to a week. The rate of change of ferrous hydroxide to 'green rust 1' is rapid, and ferrous hydroxide is not detected in the precipitate after about an hour. However the rate of change of 'green rust 1' to

Table 2. *X-ray powder diffraction data for 'green rust'*

$d_{\text{obs.}}^1$	I	(h, k, l)	$d_{\text{calc.}}^3$	$d_{\text{obs.}}^2$	I
10.92	100	00.1	10.92	11.15	10th
5.48	80	00.2	5.48	5.49	7
3.65	80	00.3	3.65	3.64	5
2.747	m	01.0	2.747	2.740	3
		00.4	2.735		
2.660	ms	01.1	2.666	2.669	6
2.459	ms	01.2	2.457	2.451	7
2.195	ms	01.3	2.196	2.192	6
		00.5	2.188		
1.938	ms	01.4	1.939	1.937	6
		00.6	1.818	1.828	$\frac{1}{2}$
1.712	w	01.5	1.712	1.712	4
1.587	w	11.0	1.587	1.587	4
1.570	w	11.1	1.571	1.570	4
		00.7	1.563		
1.525	w	11.2	1.524	1.524	4th
		01.6	1.519		
		11.3	1.455	1.454	1
		20.0	1.374	1.374	$\frac{1}{2}$
		11.4	1.373		
		00.8	1.386		
		20.1	1.364	1.359	$\frac{1}{2}$
		01.7	1.359		
		20.2	1.333	1.335	$\frac{1}{2}$
		20.3	1.286	1.286	1
		11.5	1.285		
		20.4	1.228	1.227	1th
		01.8	1.224		
		00.9	1.216		
		11.6	1.197	1.163	$\frac{1}{2}$
		02.5	1.164		
		11.7	1.114	1.099	< $\frac{1}{2}$ th
		01.9	1.112		
		02.6	1.098	1.038	$\frac{1}{2}$
		00.10	1.094		
		12.0	1.039	1.028	$\frac{1}{2}$
		12.1	1.035		
		02.7	1.032	1.0000	$\frac{1}{2}$
		11.8	1.030		
		12.2	1.021	0.9704	$\frac{1}{2}$ th
		01.10	1.016		
		12.3	0.9993	0.9704	$\frac{1}{2}$ th
		00.11	0.9714		
		12.4	0.9713	0.9650	$\frac{1}{2}$ th
		02.8	0.9694		
		11.9	0.9650		

Table 2 (cont.)

$d_{\text{obs.}}^1$	I	(h, k, l)	$d_{\text{calc.}}^3$	$d_{\text{obs.}}^2$	I
		12.5	0.9386	0.9385	$\frac{1}{2}\text{th}$
		01.11	0.9352		
		30.0	0.9163		
		30.1	0.9131	0.9161	$<\frac{1}{2}$
		00.12	0.9117		
		02.9	0.9105		
		30.2	0.9038	0.9033	$<\frac{1}{2}$
		11.10	0.9007		
(all possible reflections)					

¹ Data from BERNAL, DASGUPTA and MACKAY (1958). ms = medium strong; m = medium; w = weak.

² Data from Spec. No. 1118/0. (RICKARD). th = thick.

³ $d_{\text{calc.}}$ from $a_0 = 3.17 \text{ \AA}$, $c_0 = 10.94 \text{ \AA}$ (RICKARD).

'green rust 2' is slower and takes two to three days. Since the bacteria have a lag phase at least as long as this period, the sulphidation experiments were essentially concerned with 'green rust 2'.

'Green rust 2' was therefore examined in detail and was shown to consist of hexagonal flakes, thin enough for penetration by the electron beam of an electron microscope, but up to 10 microns in length. Because of the importance of 'green rust 2' in these experiments, d values were calculated for all possible reflections for the material, based on the data and cell size of $a_0 = 3.17 \text{ \AA}$, $c_0 = 10.94 \text{ \AA}$ given by BERNAL, DASGUPTA and MACKAY (1958). The observed values were consistent with these calculated values, as is shown in Table 2.

RESULTS

Control experiments were performed without bacteria, but under identical conditions to those of the inoculated experiments, to confirm that hydrogen sulphide could not be generated abiologically in the media, and to establish whether there was any reaction between the medium and the precipitate. In no case was any reaction observed in the controls, and therefore all the reactions recorded were a direct result of the presence of bacteria.

A summary of results from these experiments is given in Table 3.

The first iron sulphide product identified in the sulphidation of 'green rust 2' was mackinawite. This was observed two weeks after the inoculation of the medium; that is, between three and seven days after bacterial growth had started.

Bacterial growth was slow and poor under those conditions where the

Table 3. *Summary of experimental results*a) *Bacteriological sulphidation of a ferrous salt**

Initial pH	Time	Iron sulphide products
6-8	Initial ppt.	MACKINAWITE ^{1, 2} (broad lines)
6	3 months	GREIGITE (+ mackinawite) ²
7	3 months	GREIGITE + MACKINAWITE
8	3 months	MACKINAWITE
6	6 months	GREIGITE (+ mackinawite)
7	6 months	GREIGITE
8	6 months	MACKINAWITE
8	9 months	MACKINAWITE (+ pyrrhotite + greigite)

* Before the introduction of Lindemann glass capillaries, specimens were mounted on glass fibre mounts. Superficial oxidation was therefore encountered during analysis with the production of small amounts of goethite and rhombic sulphur as contaminants.

¹ Specimen analysed wet with Lindemann glass capillaries.

² Remnant 'green rust 2' present.

b) *Bacteriological sulphidation of artificial goethite*

Initial pH	Time	Iron sulphide products
6-8	Initial ppt.	MACKINAWITE ^{1, 2} (broad lines)
6	3 months	MARCASITE (+ pyrite) ^{1, 3}
7	3 months	MARCASITE + PYRITE (+ mackinawite) ^{1, 3}
8	3 months	MACKINAWITE ^{1, 3}
6	6 months	MARCASITE (+ pyrite) ^{1, 3}
7	6 months	MARCASITE + PYRITE ^{1, 3}
8	6 months	PYRITE + MARCASITE ^{1, 3, 4}

¹ Specimen analysed wet with Lindemann glass capillaries.

² Major remnant goethite still present.

³ Elemental sulphur absent from precipitate; 'oil' present.

⁴ Pyrite/marcasite partially located as thin film on tube sides and medium surface.

mackinawite-greigite transformation occurs rapidly (i.e. pH = 6.5 and below (RICKARD, 1969)). Three months from inoculation however, greigite was identified as the major product of the tubes started at pH = 7, although mackinawite was still the only product of the higher pH cultures. The same result was observed after six months ageing. However, after nine months, greigite was identified with the mackinawite precipitate, and there was also some pyrrhotite. Pyrite, marcasite and smythite were not formed during these experiments.

Mackinawite was also the primary product of the bacteriological sulphidation of goethite. In these experiments growth was observed more rapidly at the lower pH values. The reason for this was perhaps that the solubility of ferric iron in solutions at pH values of about 6 is far lower than the solubility of ferrous iron, and therefore iron toxicity, which may have affected the ferrous iron experiments, did not occur. After three months at about pH = 6, marcasite was found admixed with the mackinawite. At pH = 7, pyrite and marcasite were found admixed with remnant mackinawite, but at pH = 8 mackinawite was the sole sulphide identified. However after six months, major pyrite was discovered in the tubes which started at pH = 8.

The location of pyrite in these tubes was unusual. It was found mainly to occur on the sides of the tubes and floating on the medium surface. The reasons for this are unknown, but it might have been caused by some flotation or surface tension phenomenon occurring in the presence of large concentrations of organic matter.

Generally, reactions were slower in these experiments than in the corresponding inorganic investigation. This was caused by a complete lack of agitation of the solid phases. Goethite for example, was not completely sulphidised for three months, a reaction which was completed in less than a week in the highly-agitated abiogenic experiments.

DISCUSSION

Chemistry and structures of the biogenic sulphides

No crystallochemical differences were detected between the iron sulphides produced through purely inorganic reactions and those produced bacteriologically. Of the five iron sulphides formed during these experiments (mackinawite, greigite, pyrite, marcasite, pyrrhotite) only mackinawite was obtained pure enough to compare precisely with the inorganically-produced compounds. The other products were mixed with one or more of the other sulphides. A comparative x-ray diffraction analysis for biogenic and abiogenic mackinawite is shown in Table 4.

Chemical analyses of this material showed that it was chemically identical to inorganic mackinawite. In both cases an iron: sulphur ratio of 1:1.1 was obtained. The excess sulphur in this analysis probably came from adsorbed or coprecipitated sulphide, in a similar manner to the excess sulphide in the inorganic preparation. Furthermore, this biogenic mackinawite, together with its associated sulphide, produced greigite when heated in an evacuated sealed tube, under identical conditions to the same reaction with the abiogenic product.

Table 4. *Comparative x-ray diffraction data for biogenic and abiogenic synthetic mackinawite and natural mackinawite*

Biogenic ¹		Synthetic mackinawite Abiogenic ²		Natural mackinawite ³	
5.05	vs	5.03	vs	5.03	vs
2.99	s	2.97	s	2.97	s
2.60	mw	2.60	w	2.60	vw
2.31	s	2.28	m	2.31	s
1.833	mw	1.838	mw	1.838	m
1.803	s	1.809	vs	1.808	s
1.727	mw	1.722	mw	1.725	m
...	1.674	w
1.559	mw	1.564	m	1.562	mw
...	..	1.403	vw	1.409	w
1.298	m	1.298	mw	1.300	w
...	..	1.267	mw	1.258	mw
...	1.240	w
...	..	1.194	vw	1.190	vw
...	1.174	vw
1.127	mw	1.129	w	1.133	mw
1.052	m	1.055	mw	1.055	mw
...	1.037	b
...	1.027	b
...	1.000	b

vs = very strong; s = strong; m = medium; mw = medium weak; w = weak; vw = very weak.

¹ Mackinawite produced by the bacteriological sulphidation of goethite after three months ageing.

² Mackinawite produced through the reaction between sodium sulphide and ferrous sulphate after one week ageing.

³ KOUVO, VUORELANIAN and LONG (1963).

Since mackinawite has been shown to be a simple ferrous sulphide, the similarity of the abiogenic and biogenic forms implies that the sulphur species involved in the sulphidation process is the same from both sources. The detailed investigation of the chemistry of the formation of the iron sulphides indicated that the presence of more oxidised sulphur forms than sulphide results in the formation of iron sulphides other than mackinawite. Therefore sulphate-reducing bacteria produce only sulphide species; they do not produce any other, more oxidised, sulphur species in detectable quantities. The exact nature of the sulphide species produced depends on the pH of the medium, since, whatever the pH within the cell itself, the sulphide formed on coming in contact with the medium will change to the thermodynamically dominant species instantaneously.

Textures of the biogenic sulphides

Apart from the location and form of the pyrite and marcasite in the products of the bacteriological sulphidation of goethite, there was no evidence to suggest that the biogenic and abiogenic sulphides could be distinguished. In both cases the products were extremely fine-grained and defied any detailed optical examination.

The absence of framboids, microspheroidal aggregates of pyrite microcrysts, is of interest. This widespread texture of pyrite has long been tenuously associated with microorganic activity. However it can be stated with some confidence that the absence of such forms demonstrates that sulphate-reducing bacteria do not exert any *direct* influence on the textures of pyrite.

As mentioned above, the formation of thin films of pyrite and marcasite microcrysts on the surface of the medium and on the sides of the culture tube in contact with the medium seems to have been caused by some flotation process. Where these films of pyrite and marcasite were formed, a thick black oil-like material was found in the bottom of the culture tube. This 'oil' was a mixture of dead bacteria and their waste products with iron sulphide, which probably gave the 'oil' its black colour. The 'oil' was immiscible with the medium. The presence of this 'oil' suggests that it was probable that surface-active agents were present in the medium at that time that could have accounted for the flotation of the pyrite and marcasite.

The presence of surface-active agents in the waste products of bacteria in the natural aqueous environment could have a profound effect on the iron sulphide textures. Although framboids of pyrite were not identified in these experimental investigations, one major factor was absent which may be of great importance naturally: a slow rate of formation of the pyrite. The reactants in these experimental systems were concentrated relative to those in most natural systems. In particular it is not anticipated that modern sedimentary environments, where framboids are forming abundantly, would frequently have concentrations of iron and sulphide approaching one molar. Therefore the reaction rate in the natural aqueous environment would be slow relative to that encountered in the laboratory. The subsequent slow production of pyrite from the initial mackinawite (assuming that a ferric oxyhydroxide is being sulphidised), under the influence of surface-active agents from the autolysed bacteria and their waste products, may result in the formation of framboidal forms of pyrite.

Little is yet known about the reactions between the complex organic compounds associated with bacteriological environments and iron sulphides, and it seems that this field of research may give rise to important sedimentological results. It has been shown, for example, that pyrite is relatively soluble in solutions of amino acids (NEUBERG and MANDL, 1948). The effect of low concen-

Chemical aspects of the formation of biogenic sulphides

The initial product of the sulphidation of both the ferrous salt and goethite was mackinawite. This is in accordance with the results from inorganic experimentation (RICKARD, 1969 and BURKIN and RICKARD, 1969). Greigite was formed from mackinawite by a further reaction with sulphide both in the medium and by heating dried material. The reaction was faster at lower pH values. These reactions have been observed inorganically. The formation of

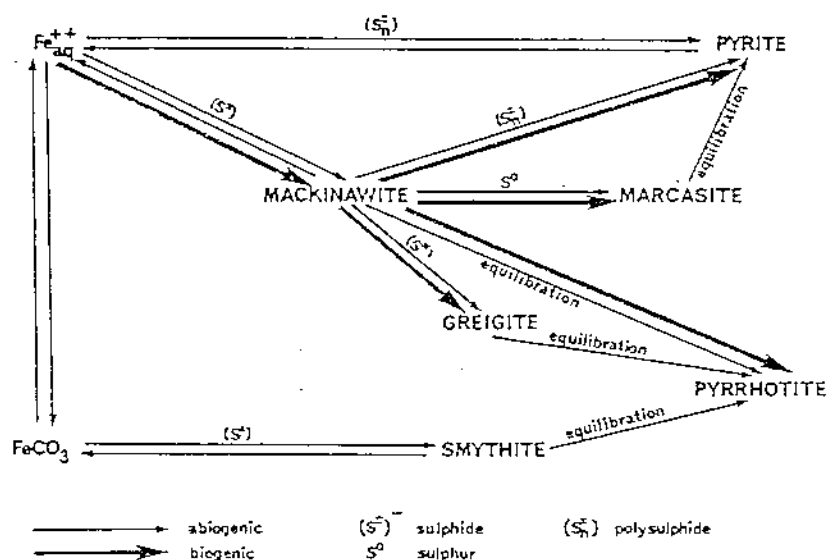


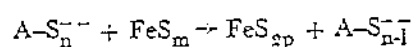
Fig. 2. Summary of the major iron sulphide interrelationships in aqueous solutions and their biogenic counterparts observed in this investigation (reactions involving ferric iron not included).

pyrite and marcasite reflects closely the conditions under which these compounds were formed abiogenically; that is by re-reaction between the sulphur, polysulphides and mackinawite resulting from the sulphidation of goethite. As in the abiogenic experiments marcasite was formed at more acid pH values.

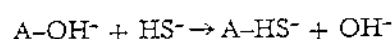
One major, and possibly extremely important, indirect difference between the abiogenic and biogenic experiments, was the absence of rhombic sulphur admixed with the iron sulphides in the sulphidation of goethite, in all but the initial products of these experiments.

Although the conditions in the culture media were such that sulphur is not thermodynamically stable, experience with inorganic systems showed that the rate of dissolution of sulphur is slow at pH values below 7. Furthermore, as has been mentioned above, the reaction rates were generally lower in these biogenic experiments than in the corresponding inorganic systems, because of the absence of agitation. The relatively rapid disappearance of sulphur indicates that, in these cultures, reactions involving sulphur took place faster than was observed in the absence of bacteria. It is known that polysulphide groups can be attached to a number of complex inorganic compounds (PRYOR, 1962), and the presence of large quantities of such compounds, in the form of the 'oil' mentioned above or dissolved in the medium, from the autolysis of bacterial cells and mucin, may have some catalytic effect on the dissolution of sulphur.

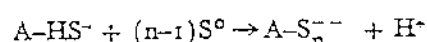
Reactions of the form:



(where A is the organic compound, m is mackinawite and p is pyrite) may be important in the formation of pyrite bacteriologically. The compound $A-S_n^{--}$ is formed from exchange and oxidation reactions of the type:



and



Furthermore sulphur could react directly with organic compounds to form organic-sulphur complexes, which, on reacting with mackinawite, form marcasite.

In both cases the net result would be the apparent disappearance of sulphur and the formation of marcasite and pyrite. The effect of the organic compound is essentially catalytic.

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